

AMENDMENTS TO THE SPECIFICATION

Please replace Paragraphs [0029], [0038], [0049-0052], [0054], [0056], and [00105] with the following paragraphs rewritten in amendment format:

[0029] (Fig. 19) Detailed figure of Vaccibody gene construct. The targeting unit is inserted between the BsmI/MfeI and BsiWI restriction enzyme sites (The V cassette of the pLNOH₂ vector). The hinge-Cy3-Fv³¹⁵ is inserted between the HindIII and BamHI sites into the C cassette of pLNOH₂. The hinge and the Cy3 domain as well as the two scFv's are connected with (G₄S)₃ linkers (black boxes). The Cy3 and the Fv³¹⁵ are connected through a GLSGL linker (SEQ ID NO:41). The Fv³¹⁵ is inserted between two nonidentical SfiI restriction enzyme sites. The antigenic unit and dimerization motif may be of any origin appropriate. Also, functional fragments of Cy3 may be employed, or a sequence which is substantially homologous to the Cy3 sequence or Cy3 fragments. In relation to Figures 8 and 9, the peptide linker sequence comprises GGGSSGGGSSGGSS (SEQ ID NO:42). In relation to FIG 10 and 11, linker sequence shown comprises GGGGSGGGGSGGGGS (SEQ ID NO:43).

[0038] The crucial dimerization motifs in the Vaccibodies constructed in the examples so far, include hinge regions and Cy3 domains. The hinge contributes to the dimerization through the formation of interchain disulfide bridges. In addition, it functions as a flexible spacer between the domains allowing the two scFvs with targeting tasks to bind simultaneously to two target molecules expressed with variable distances (Fig 2).

The C α 3_Cy3 domains contribute to the dimerization through hydrophobic interactions. These dimerization motifs can be exchanged with other multimerization moieties (e.g. from other Ig isotypes/subclasses).

[0049] The gene for the hIgG3 hinge and CH3 domain was cloned from the pUC19 vector containing hinge genetically combined with Cy3 genes of the hIgG3 subclass (Olafsen, Rasmussen et al. 1998). Two variants of the hinge length in the humanized Vaccibodies were made; one with just the h4 exon connected to the CH3 domain (sh) and one with both exon h1 and h4 connected to the CH3 domain (lh) (Fig 8). The primers included restriction enzyme sites (underlined): 5'h4: tag caa gct tgg cca gcg cag gga g (SEQ ID NO:1); 3'CH3: cag gcc acc gag gcc ttt acc cgg aga cag gga (SEQ ID NO:2). The h1 exon were introduced directly upstream of the h4 exon by QuickChange PCR using these primers Qh1a: ctccaatcttctctctgca **gag ctc aaa acc cca ctt ggt gac aca act cac aca** gag ccc aaa tct tgt gac ac (SEQ ID NO:3) and Qh1b: gt gtc aca aga ttt ggg ctc **tgt gtg agt tgt gtc acc aag tgg ggt ttt gag ctc** tgcagagagaagattgggag (SEQ ID NO:4).

[0050] The murine Vaccibodies have a complete hinge and CH3 domain of the mIgG2b subclass picked up by PCR from a pUC18 vector containing the Cy2b genes (Fig 9). The primers included restriction enzyme sites (underlined) or linkers (bold) with the complementary sequences (italic): 5'hinge: tagcaagctt ca gag ccc agc ggg ccc (SEQ ID NO:5); 3'hinge: 5'**tcc acc tcc gct gct tcc acc gcc** tgg gca ttt gtg aca ctc ctt g (SEQ ID NO:6); 5'CH3: **gga agc agc gga ggt gga agt gga** ggg cta gtc aga

gct cca ca (SEQ ID NO:7); 3'CH3: cag gcc acc gag gcc acc cgg aga ccg gga gat g (SEQ ID NO:8). The hinge and the CH3 domain were then joined by PCR SOEing.

[0051] The Antigenic V region genes were cloned from the plasmacytoma MOPC315.4 (Eisen, Simms et al. 1968). The V regions were obtained by extracting mRNA from the MOPC315.4 cell line with oligo (dT)-coated magnetic Dynabeads (Dyna). First strand cDNA were then made and used as template for PCR amplification of the V region genes using specific primers annealing to the exact ends of the M315 V region sequences. The primers included restriction enzyme sites (underlined) or linkers (bold) with the complementary sequences (italic). The primer sequences were: 5'VH: ggc ctc ggt ggc ctg gat gta cag ctt cag gag tca (SEQ ID NO:9); 3'VH: **gcc aga gcc acc tcc gcc aga tcc gcc tcc acc** tga gga gac tgt gag agt ggt (SEQ ID NO:10); 5'VL: **ggc gga ggt ggc tct ggc ggt ggc gga tcg** cag gct gtt gtg act cag gaa (SEQ ID NO:11); 3'VL: gacg tcgac tag gac agt gac ctt ggt tcc(SEQ ID NO:12). The VH and VL genes were then joined by PCR soeing to a scFv format (Fig 10).

[0052] The complementary sequences in the tags 3' of the Cy3 coding region and 5' of the M315 VH coding region enabled the M315 scFv to be combined with the three different hinge-CH3 genes by PCR SOEing (Fig 11). The products of this reaction were then digested with HindIII and Sall and subcloned into a pUC19 vector (Fig 12). Two BamHI restriction enzyme sites inside the V regions of M315 were removed by QuickChange PCR (Fig 13) using primers: BamHI VL1:at gcc aac tgg ata caa gaa aaa cc (SEQ ID NO:13); BamHI VL2: gg ttt ttc ttg tat cca gtt ggc (SEQ ID NO:14) at, BamHI

VH1: tgg aac tgg ata cgg cag ttt cc (SEQ ID NO:15) and BamHI VH2: gg aaa ctg ccg tat cca gtt cca (SEQ ID NO:16). A following QuickChange PCR using primers: 3'VL stop1: gtc act gtc cta tga ggctgcagggcc ggatcc gtcgactctag (SEQ ID NO:17) and 3'VL stop2: cta gag tcg ac ggatcc ggccctgcagggcc **tca** tag gac agt gac (SEQ ID NO:18), were then performed to introduce a stop codon (bold), a SfiI and a BamHI restriction enzyme site (underlined) downstream of the coding region (Fig 14).

[0054] The V region genes providing specificity for MHC class II had previously been cloned from the 14-4-4S hybridoma (Lunde, Western et al. 2002), which produces an Ab specific for the Eα chain (determinant Ia.7) of the I-E MHC class II molecule (Ozato, Mayer et al. 1980). Specific primers annealing to the exact ends of the V region sequences with tags designed to include restriction enzyme sites (underlined) or linker sequences (bold) with the complementary sequences (italic). The primer sequences were: 5'VL: gac att caattg *aca* cag tct tct cct gct tcc (SEQ ID NO:19); 3'VL: **gcc aga gcc acc tcc gcc aga tcc gcc tcc acc** ttt gat ttc cag ctt ggt gcc (SEQ ID NO:20); 5'VH: **ggc gga ggt ggc tct ggc ggt ggc gga tcg** cag gtc cag ctg cag cag t (SEQ ID NO:21); 3'VH: ga cgtacg actcacc tga gga gac ggt gac tga gg (SEQ ID NO:22). The V region genes giving specificity for the hapten NIP (Neuberger 1983) were designed with the similar tag sequences except for the 5'VL primer: 5'VL: ggtg tgcatcc cag gct gtt gtg act cag gaa (SEQ ID NO:23); 3'VL: **gcc aga gcc acc tcc gcc aga tcc gcc tcc acc** tag gac agt cag ttt ggt acc t (SEQ ID NO:24); 5'VH: **ggc gga ggt ggc tct ggc ggt ggc gga tcg** cag gtc caa ctg cag cag cc (SEQ ID NO:25); 3'VH: ga cgtacg a ctc acc tga gga gac tgt gag agt ggt (SEQ ID NO:26). The VL and VH were then joined

by PCR SOEing (Fig 16) and subcloned into the V cassette pLNOH₂ vector containing the hinge-CH3-scFvM315 genes (Fig 17 and Fig 18). Likewise, other V genes conferring a desired specificity are isolated from hybridomas or from phage selected from phage display libraries. They are then PCR amplified using primers designed in the same manner as above and subcloned after PCR SOEing in the targeting-cassette (Fig. 17 and 18). Rearranged V_H and V_K genes conferring specificity for HLA-DP were PCR amplified from cDNA from the 22C1 hybridoma, which produces an antibody with pan HLA-DP specificity. The V genes were reamplified with new primers containing sites for direct cloning into the expression vectors pLNOK and pLNOH₂ (Norderhaug and Olafsen, 1997); 5'-VL, ggtgtgcattccgacattgtgctcacc (SEQ ID NO:27); 3'-VL, cgtacgttctactcacgtttattccagct (SEQ ID NO:28); 5'-VH, gtgcattccgaggtgcagctgcaggagtct (SEQ ID NO:29); 3'-VH, cgtacgactcacctgaggagaccgtagc (SEQ ID NO:30). Furthermore, scFV was generated by PCR SOEing using the following primers:

5'VL, g gtg tgcattc cga cat tgt gct cac c (SEQ ID NO:31)

3'VL: **gcc aga gcc acc tcc gcc aga tcc gcc tcc acc gtt tta ttt cca gct**
(SEQ ID NO:32)

5'VH: **ggc gga ggt ggc tct ggc ggt ggc gga tcg gag gtg cag ctg cag**
gag tct (SEQ ID NO:33)

3'VH, cgtacg act cac ctg agg aga ccg tag c (SEQ ID NO:34)

[0056] The chemokine genes were cloned from thioglycolate stimulated peritoneal macrophages. 4ml 2% thioglycolate were injected i.p. into Balb/c mice. 3 days later peritoneal macrophages were collected and mRNA was extracted with oligo

(dT)-coated magnetic Dynabeads. First strand cDNA was made and used as template for PCR amplification of chemokine genes (RANTES and MIP-1 α) using specific primers: 5'MIP-1 α : ggtg tgcattc cgc gcc ata tgg agc tga cac(SEQ ID NO:35), 3'MIP-1 α : ga cgtacg act cac ctg cat tca gtt cca ggt cag tg (SEQ ID NO:36) 5'RANTES: ggtg tgcattc c gcc tca cca tat ggc tcg g (SEQ ID NO:37) 3'RANTES: ga cgtacg a ctc acc tga cat ctc caa ata gtt gat gta ttc (SEQ ID NO:38). The different targeting unit genes were then digested with MunI and BsiWI or BsmI and BsiWI, respectively and subcloned into the V cassette pLNOH₂ vector containing the hinge-CH3-scFvM315 genes (Fig 17 and Fig 18). CD40 ligand is cloned from T cells that are activated with LPS for 4 hours before mRNA is extracted for preparation of cDNA. The cDNA is used as template in a PCR reaction with primers specific for the CD40 ligand sequence. Furthermore, this sequence is reamplified with primers designed to facilitate subcloning in the targeting cassette as described above.

[00105] Bone marrow aspirate from patients suffering from multiple myeloma can be collected. The mononuclear cells (MNC) can be separated using a density gradient solution of Ficoll-Isopaque (LymphoprepTM from Axis-Shield PoC AS). Total RNA can be isolated using TRIZOL® (TRIZOL[®]) Reagent from InvitrogenTM Life Technologies) from MNC, and cDNA can be made from mRNA (First-Strand cDNA Synthesis Kit from Amersham Biosciences (Not I-d(T)18 bifunctional primer)). This cDNA can be used as template in PCR with primers that amplify the V genes of the heavy or light chain of the multiple myeloma Ig. The sense primers are family specific and localized in the leader regions (VH1-7, VK1-6 and VL1-10), and the anti-sense

primers are localized in the first part of the C regions (one primer each for IgG, IgA, kappa and lambda). PCR products can be ligated into a vector (PGEM®-T (pGEM[[®]]-T Easy Vector from Promega), and transformed into E.coli. DNA samples isolated from individual colonies can be sequenced. Getting the same sequence from three different colonies originating from three different PCRs confirms that the V regions from the myeloma Ig have been isolated. PCR SOEing can be performed and reamplification is done with primers including tags with sites for Sfil as described in Figure 19. For one patient such primers had the sequence:

5'TAVH

5' ACGTAGGCCTCGGTGGCCTGCAGATCACCTTGAAGGAGTCT (SEQ ID NO:39)

3'TAVK

5'GATCCGGCCCTGCAGGCCTCATTTGATCTCCAGCTTGGTCCC (SEQ ID NO:40)